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Using stable isotopes to determine mineral bioavailability of functional foods

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Abstract: Mineral stable isotopes allow for the direct assessment of mineral absorption in all population groups. The most widely used in human nutrition are calcium, magnesium, zinc and iron. These can be used to demonstrate the effects of interventions, including the use of functional foods, on mineral absorption both from an individual meal and from whole diets. Methodologies are safe, practical and well tolerated, even by small children.

Key words: calcium absorption, iron, zinc, stable isotopes, mineral metabolism.

16.1 Introduction

16.1.1 What are mineral stable isotopes and which minerals can be studied?

Mineral stable isotopes are naturally occurring, non-radioactive forms of minerals in which the lower abundance atomic mass fractions have been enriched so that they may be used to trace the metabolism of the native compounds. By enriching what are usually rare isotopes, followed by administration of small amounts of these enriched isotopes to human or animal subjects, the rate at which these minerals enter the body or are distributed and excreted can be determined. In nutritional research, by far the most commonly studied minerals are calcium, iron, zinc and magnesium. Some research has been done with copper and selenium. Key additional minerals, most notably phosphorus, do not have any naturally occurring low abundance isotopes and thus cannot be studied using stable isotope techniques. The use of mineral stable isotopes has largely, but not completely, replaced the use of radioactive isotopes in human nutrition research (Abrams, 1999).

16.1.2 How are mineral stable isotopes produced, obtained and tested for safety?

Stable isotopes are usually obtained as the metal, oxide, carbonate or chloride forms. Enriched isotopes are produced by a number of methods described elsewhere (Yergey and Yergey, 1997; Abrams, 1999). Currently, virtually all of the mineral stable isotopes are produced either via the calutron facilities in Russia (calcium and magnesium) or via European gas centrifugation facilities (iron and zinc). The isotopes need to be converted into a soluble form, usually the aqueous chloride or sulfate prior to administration. All chemicals used in isotope preparation and sample preparation must be high purity to avoid any trace contaminants. The soluble isotope is diluted to the desired concentration, or further preparation is conducted if necessary, depending on the mineral and the form in which it is to be administered. If the isotope is to be administered intravenously, the solutions need to be sterile-filtered, packaged into vials and tested for sterility and pyrogenicity before human or animal use. This is usually done by a licensed pharmacy experienced in these procedures. Unfortunately, in the United States there are few such pharmacies and this can be an issue in obtaining isotopes prepared for human use. As with all medications, stable isotopes must be prepared and administered in accordance with accepted pharmaceutical standards. There is no intrinsic risk to the isotopes as they are naturally occurring and as currently provided by reputable dealers, are free of contamination and can usually be provided at the desired enrichment.

Mineral stable isotopes are not usually purchased directly from the manufacturers. Instead they are purchased from reputable isotope sales companies. In the past, inappropriate sales and distributions of inadequately tested materials were common problems. However, at the present time, this is not a substantial problem and with appropriate diligence, there should be no concern about the availability or safety of purchased mineral isotopes. Typical isotopes used are listed in Table 16.1. With the exception of ^{46}Ca, isotopes are usually purchased at >80% purity, often >95%. This decreases problems with using multiple isotopes of the same mineral as there is little cross-over of isotope. A unique exception is ^{46}Ca with a natural abundance of 0.003%. This isotope is usually purchased at 5–10% purity, although a maximum of about 30% has been reached. This is not problematic as extremely small doses of ^{46}Ca are used in studies.

16.1.3 What are the approximate costs of mineral stable isotopes and how are they analyzed?

The costs of a mineral stable isotope study depend on the mineral being studied, the type of study being done (absorption or absorption and kinetic turnover rate study), the size of the subjects and their age. In the case

Table 16.1 Mineral stable isotopes frequently used in nutritional research

Isotope	Natural abundance (%)	Typical dose in adults (absorption)
^{42}Ca	0.65	1–2 mg intravenously
^{44}Ca	2.08	10–15 mg by mouth
^{57}Fe	2.14	6–8 mg by mouth
^{58}Fe	0.29	3 mg by mouth
^{25}Mg	10.0	15–20 mg intravenously
^{26}Mg	11.0	25–30 mg by mouth
^{67}Zn	4.1	2–4 mg by mouth
^{70}Zn	0.6	0.4–0.5 mg intravenously

Typical doses are based on dual tracer studies involving adults and children over 12 years of age. Other isotopes may be chosen and dosing depends on physiological conditions, expected absorption and analytical methodology/precision.

of calcium, for example, the largest isotope doses must be used on adolescents due to their high rates of bone turnover. For iron, as the tracer is primarily distributed into red blood cells, the whole body quantity of which is body weight dependent, it is usually less expensive to study small children than adults. Studies in which it is expected that absorption will be low due to dietary inhibitors or subject characteristics would need a greater dose. Ultimately, it is the precision of the measurement equipment being used for each isotope that determines how much the isotopes need to be enriched in the body fluid sample to be measured and thus what dosing is needed.

Analytical techniques for isotope analysis for minerals are very different from those for organic substances or other elements such as oxygen and nitrogen (e.g. deuterium). Briefly, most analysis are currently undertaken using either thermal ionization mass spectrometry or inductively coupled plasma mass spectrometry. These techniques are specialized and generally available only at a small number of institutions, as discussed below.

However, with these caveats in mind, it is not uncommon for the isotope costs for a single measurement of calcium, iron or zinc absorption to be between $100 and $200/subject. Magnesium measurements can be about twice this expensive. The cost of analysis is dependent on the mineral, the type of equipment used, the precision needed and the general operating characteristics of the laboratory. Costs typically range from $50 to $200 for a sample analysis in laboratories that process a large number of nutritional samples. Some laboratories may change considerably more for small sample volumes or high-precision measurements.

16.2 Methodological issues in using stable isotopes to study human nutrition

The methodologies used in mineral stable isotope studies are primarily mineral specific, rather than food specific. By this it is meant that very similar methods are used to evaluate the effects of diet and dietary interventions using stable isotopes regardless of the food or meal matrix in which the mineral of interest is found (Tables 16.2 and 16.3). What is different in how the research is conducted is which minerals are being evaluated. Calcium, magnesium, zinc and iron methodologies will be considered below.

16.2.1 Calcium

There are six naturally occurring stable isotopes of calcium. Choice of isotopes and dosing levels depends greatly on the type of study being conducted (i.e. absorption or kinetic), the likely level of mineral absorption and the type and precision of analytical technique being used (Abrams, 1999). The method used for most current studies for calcium (and magnesium and zinc) is the dual-tracer method. In this method, one isotope (e.g. ^{44}Ca) is given by mouth while a second enriched isotope (e.g. ^{42}Ca) is given intravenously. The relative enrichment of the oral versus the intravenous (IV)

Table 16.2 Minerals and preferred collection methods

Mineral	Dosing method	Collection method
Calcium	IV and oral	24–48 hour urine
Zinc	IV and oral	Spot urine 72–96 hours
Magnesium	IV and oral	Complete 72 hour urine
Iron	Oral	Red blood cells 14 days

Table 16.3 Guidelines for obtaining and preparing isotopes

Purchasing
- Experienced dealer
- Provision of complete tracking of isotopes from source
- Certified analysis
- Pricing based on volume and enrichment

Testing and preparation
- Certification by dealer
- Compounding by licensed pharmacist
- Sterility and pyrogenicity testing as needed
- Testing of final compounds for total mineral and isotopic enrichment

tracer in an appropriate body fluid is a measure of the absorption over 50 years ago (Bronner and Harris, 1956).

Early in the morning of the study, subjects are instructed to empty their bladders, and are given breakfast. Toward the end of the meal, the subjects are given an isotope of calcium that has been premixed (and allowed to equilibrate in the refrigerator for 12–24 h) with milk or juice. After breakfast, a different calcium isotope is administered intravenously over 2–3 min (Abrams *et al.*, 1997).

After administration of the tracers, a complete 24 h urine collection is carried out. The relative fraction of the oral vs. the IV tracer dose in this 24 h urine pool is determined and represents the fraction of the oral tracer dose that was absorbed. Spot determinations of urine or serum isotope levels of the tracers may also be used. However, for calcium, this method may not be as accurate as that determined from complete 24 h collections (Yergey *et al.*, 1994). Calcium is extracted from the urine sample via oxalate precipitation. For magnesium, iron and zinc, ion exchange columns are needed. Published methods are available for each of these minerals. In general, it is best to have the sample preparation done at the laboratory performing the mass spectrometric analysis.

In studies in adults, it may be more convenient to obtain a single timed blood sample rather than a 24 h urine collection. In this variation of the method described above, the oral isotope is given about 2 h before the IV isotope. Then a single blood sample is obtained usually 3–4 hours after the IV dose. The relative enrichment of the oral versus the IV tracer in the blood is used to calculate absorption. In theory, this method may be slightly less accurate than the urine method due to variable rates of absorption of the oral tracer. However, in practical terms, they appear to give very similar results and either method may be used in research studies (Abrams, unpublished observations). When used for clinical purposes on an individual patient, the dual-tracer method with 24 h urine collection would remain the gold standard at this time.

In studies in adults, some have used only a single oral tracer and estimated the IV results using height and weight of the subject (Heaney *et al.*, 2002). In general, this approach is less preferred than the dual tracer approach, especially for clinical use or smaller research studies. The IV tracer is a relatively small part of the overall study cost and is safely administered without difficulty in adults. Studies using only an oral tracer are likely to give accurate answers in relatively large sample groups of adults, but more reliance can be given to those conducted using both an oral and an IV tracer.

An important variation in this methodology was necessary for studies of prebiotics and calcium absorption. It was observed from some studies that a benefit was seen in some cases and not in others (Griffin and Abrams, 2005). A key feature of studies that did not show a benefit was that the 24 h urine method had been used. Since prebiotics primarily increases calcium

absorption by increasing calcium absorption in the colon, using a 24 h urine collection may be inadequate to identify this effect. Instead, collecting all urine for a total of 36–48 h after dosing may be needed to evaluate factors which affect calcium absorption by the colon. Therefore, when evaluating dietary factors which may alter the site of calcium absorption, a 48 h urine collection should be performed rather than a 24 h collection to be certain of capturing this effect (Abrams *et al.*, 2007).

16.2.2 Magnesium

There are only three naturally occurring isotopes of magnesium, none of which is a true low-abundance one. This means that higher doses must be used than for other minerals and the relative cost is greater, as is the analytical challenge. Nonetheless, magnesium stable isotope studies are quite feasible and have been used in the evaluation of prebiotics (Holloway *et al.*, 2007).

The methods used in studies of magnesium absorption are extremely similar to those used for calcium with one important difference. That difference, identified in early studies, is that magnesium absorption is a slow process and collection of urine for a full 72 h after ingestion of the oral isotope is needed to measure absorption. Therefore, in studies of magnesium absorption it is necessary to collect urine for 3 days after dosing (Abrams *et al.*, 1997; Abrams 2003). This is challenging for many people due to work and schooling issues. The reliability of a 3-day urine collection may be less as well. Nonetheless, this method has been successfully used in studies involving both children and adults.

16.2.3 Zinc

There are five naturally occurring stable isotopes of zinc. Two are widely used in clinical studies (^{67}Zn and ^{70}Zn) and a third (^{68}Zn) can be used in some situations. Assessment of zinc isotopes in blood and urine is one of the more readily performed mineral stable isotope analysis by mass spectrometry. Recognition of the crucial role of zinc in promoting health has led to zinc becoming one of the most widely studied minerals in nutritional sciences at this time. Isotope-based studies have often evaluated factors such as the interaction of zinc and iron on the absorption of these minerals. Extremely few studies have evaluated the effects of food or diet on zinc absorption in humans. It is very likely, however, given the high frequency of zinc deficiency and the need to provide more enhanced methods of zinc delivery, that this type of research will increase.

Methodologically, there are two important issues differentiating zinc from calcium and magnesium research studies. The first is that it generally is not necessary to collect a complete 24–72 h urine after dosing of the isotopes. A spot urine sample, collected at least 72 h after dosing, is usually

adequate. This spot method is much more reliable for zinc than for calcium or magnesium which simplifies the clinical protocols (Friel *et al.*, 1992; Griffin *et al.*, 2004).

A second, more difficult issue is related to determining what information is obtained from isotopic zinc absorption studies based on the regulation of zinc absorption. This requires consideration of what is actually meant by the term 'absorption'. Absorption, as calculated based on the difference between enteral intake and fecal excretion of a nutrient, consists of two components. The first is the quantity of material that is absorbed from the diet and enters the bloodstream via the gastrointestinal tract. This is referred to as 'true' dietary absorption. The other is the total difference between the amount of a nutrient in the diet and the amount that is recovered in the stool. This difference is referred to as 'net' absorption. These two 'absorptions' are different because some of what is excreted in the stool does not come from a particular meal that is traced, but from secretion of nutrients into the gastrointestinal tract (Abrams *et al.*, 1991). This additional loss of a nutrient is not measured by dual tracer studies in which urine or blood are collected as described above.

For some nutrients, this additional nutrient loss, called 'endogenous secretion', is not a major factor in the regulation of the nutrient and can either be ignored as it is very small (e.g. magnesium) or estimated for other purposes based on dietary intake (e.g. calcium) (Abrams *et al.*, 1991). Zinc, however, is unique in that nutrient regulation is significantly related to endogenous secretion and failure to measure this may not provide a complete picture of the effects of diet on zinc homeostasis (Griffin *et al.*, 2004).

If the research question being asked is specific to the bioavailability of the source of zinc and its solubility and dietary absorption, then the dual-tracer method with urinary assessment of absorption will provide the desired answer. If, however, the dietary change considered may affect gut function and the research question is to evaluate these effects, then endogenous secretion needs to be measured. For example, if one is comparing the absorption of zinc oxide and zinc gluconate in a food source, then the dual tracer method with urinary excretion measurement of absorption will distinguish between the absorption of these two zinc sources. If, however, one is looking at whether prebiotics or probiotics beneficially affect the gastrointestinal tract in people with inflammatory bowel disease and thus lead to less zinc wasting, then the urinary method will not be adequate for that assessment of absorption.

It is possible to directly measure the rate of endogenous secretion of minerals with tracers and thus answer these questions. Unfortunately, this is most accurately done with fecal collections. Fecal collections are difficult and cumbersome to obtain, process and analyze, but would be needed for some types of zinc-related research. Further details are provided elsewhere related to this issue (Abrams, 1999; Griffin *et al.*, 2004).

16.2.4 Iron

Iron presents different issues in measuring dietary bioavailability. There are four naturally occurring iron stable isotopes. The lowest-abundance isotopes, ^{58}Fe and ^{57}Fe, are most commonly used in human nutrition research. Iron stable isotopes are usually converted to ferrous sulfate prior to oral administration. Because dosing of iron stable isotopes is dependent on enriching the circulating body iron pool, the dose administered is usually dependent on the subject's weight, and increases in proportion to weight and hemoglobin concentration (Abrams *et al.*, 1994).

The methods for iron isotope studies are somewhat different from those for the other minerals. In general, the incorporation of a dietary iron dose into the red blood cells (RBC) is measured by collecting a single blood sample, usually 14 days after an oral dose and determining the isotopic enrichment relative to the total body circulating iron. This requires measuring hemoglobin concentration and estimating blood volume. In healthy children after the first year of life, about 80–90% of absorbed iron will appear in the red blood cells by 14 days. However, in special circumstances, including children with anemia associated with inflammatory diseases and infants, this percentage may be much lower. The actual percentage of absorbed iron that can be found in red blood cells can be measured by giving an iron stable isotope intravenously and determining the incorporation of this isotope into red blood cells 14 days later (Ames *et al.*, 1999). However, this method may not accurately reflect dietary iron handling and its use is limited because of safety concerns related to infusing iron intravenously.

For practical purposes, an intravenous tracer of iron is rarely administered. Commonly, however, one dose of iron isotope is administered orally with a meal or the form of iron being investigated and a second dose of a different isotope administered after an overnight fast with ascorbic acid. This second dose is referred to as a 'reference dose' and its absorption reflects the patient's iron status. Red blood cell incorporation of this dose is usually well correlated with serum ferritin or other iron status markers (Abrams *et al.*, 1994; Abrams, 1999).

Because the use of ^{57}Fe in larger children (>about 40 kg) or adults generally requires a dose of at least 6–8 mg, it is often given split over 2 days so that the total dose on any given day does not represent too large a proportion of usually daily iron intake. In most studies, a maximum of 4 mg of iron is given as the isotope in a 24 h period. Similar concerns are not as great for the other minerals as oral isotope doses are usually <10% of typical daily intake.

16.3 Study example: enhancement of calcium absorption by prebiotics in adolescents

Several studies have evaluated the use of stable isotopes to assess the effects of prebiotics on calcium absorption. We conducted a study using

both calcium absorption and bone mineralization as outcomes. This study and its findings, especially methodological issues, will be considered in this section as an example of how the stable isotopic methodologies described in this chapter are used to evaluate functional foods (Abrams *et al.*, 2005).

16.3.1 Study design

We conducted a study using healthy young adolescents in which we randomized 100 subjects in a double-blinded fashion, stratified by gender to one of two carbohydrate supplement groups; either 8 g/day oligosaccharides of an inulin-type fructan, or maltodextrin placebo. Subjects mixed the carbohydrate supplement with calcium-fortified orange juice and drank it with breakfast daily for 12 months. After 8 weeks of receiving the carbohydrate supplement, subjects returned for a repeat calcium absorption study. Twelve months after the initial baseline study, they returned for a follow-up visit in which measurements of calcium absorption and bone mineralization were performed.

Subjects received a breakfast that contained approximately one-third of their daily intake of calcium (including the tracer-containing juice). Toward the end of breakfast, subjects were given 20 μg of ^{46}Ca which had been mixed with 240 mL of calcium-fortified orange juice. After breakfast, ^{42}Ca (1.2 mg) was infused over 2 min via a heparin lock catheter. Beginning with breakfast, a complete 48 h urine collection was obtained. Calcium absorption was calculated from the relative recovery of the oral and the intravenous tracer during the 48 h study period.

Two methodological issues can be commented upon. The first is the decision to give the calcium stable isotope once during the day with breakfast. An alternative approach would have been to determine the usual distribution of calcium in each subject's diet and then distribute the isotope proportionally between those multiple meals. Using multiple isotope doses may more accurately reflect the isotope distribution during a day. However, even that cannot completely reflect the distribution of calcium in the daily diet and each time that the isotope is mixed with food leads to potential dosing errors and inaccuracies. In general, we prefer to limit to one dose for each study and find this to provide adequate information about calcium absorption over the course of a day.

The second issue is the choice of material to which to add the tracer. It is important to be consistent in doing studies in individuals and therefore most groups have used fixed meals to which the tracer is added. However, for studies in subjects that have very low or very high usual calcium intakes, this can be problematic. For example, if a subject has a usual calcium intake of <500 mg/day and does not drink milk or calcium-fortified beverages, then it can be very difficult to accurately assess their usual calcium absorption using a fixed meal single-dose approach. That is because most of their daily

calcium may be associated with vegetables or other non-dairy calcium sources for which the extrinsically administered isotopes, especially when given with only one meal, do not accurately reflect usual absorption (Heaney *et al.*, 1990, 2000).

16.4 Results from this study

After adjustment for ethnicity, gender, *FokI* genotype, calcium intake at each visit and Tanner stage at enrolment, the effect of the prebiotic on fractional absorption of calcium was significant ($p = 0.02$) (Abrams *et al.*, 2005).

We further evaluated the results for calcium absorption at 8 weeks and 1 year relative to the baseline absorption values. These results demonstrated significantly greater calcium absorption at 8 weeks (difference 8.5 ± 1.6%, $p < 0.001$) and 1 year (difference 5.9 ± 2.8%, $p = 0.04$) for prebiotic subjects compared with control subjects. Similarly, both whole body bone mineral content (BMC) and whole body bone mineral density (BMD) were increased at one year in those receiving the prebiotic. For whole body BMC, the increase in BMC was 245 ± 11 g/year vs 210 ± 10 g/year for the control ($p = 0.03$). For whole body (areal) BMD, the increment was 0.047 ± 0.004 g/cm^2/year for the prebiotic group versus 0.032 ± 0.004 g/cm^2/year for the control group ($p = 0.01$).

An important methodological point related to these studies is the feasibility of performing repeat studies on the same individuals. Limitations to this can come in several areas. First, is the concern that isotope from the previous study will still be in the urine or blood at the time of the subsequent study limiting the ability to repeat studies. This is a complex issue, but this problem is dependent on the isotopes and the turnover rate of the minerals. For calcium, we have found that a time period of 2 weeks between studies readily allows for a repeat study. In adults, but not generally in children (because of slow bone turnover in adults), a baseline sample would be needed before a second study spaced apart by less than about 6 weeks. Using this baseline sample, we can correct for the small amount of 'residual isotope' in present at baseline. For zinc and magnesium, a similar approach can be taken, although experience is more limited with the time needed between studies.

The most important issues related to this are for studies of iron. Unlike the other minerals which are deposited in tissues or excreted, iron, once absorbed, remains in a relatively fixed pool of RBCs. Once dosed, it is possible to measure residual iron isotope in a sample of RBCs years after initial dosing. Furthermore, although most absorption and incorporation occurs within 14 days after dosing, in infants this can take longer and the levels do not stay constant. These issues can be managed by measuring the baseline levels of isotopes in the blood between studies but we tend to be cautious

about multiple iron studies in small children and avoid them when possible.

16.5 Sources of further information and advice

16.5.1 Finding laboratories to perform mineral mass spectrometry analyses

Mineral stable isotope analyses are commonly performed either in specialized nutrition laboratories experienced in these analyses. In the United States there are about eight to ten such laboratories routinely performing these analyses. Most or all of these groups will perform 'outside' analyses for nutrition investigators, but their capacity to do so is often limited. Some may be able to only analyze one mineral such as zinc, whereas others will do all of the minerals described here.

An alternative source for mineral mass spectrometric analysis is a geology laboratory that performs these analyses. Many of these laboratories routinely perform analyses of lead and other minerals not of nutritional interest. However, a large number of laboratories in geology research have the technical capacity to measure iron, zinc, calcium and magnesium. Although this can be a useful approach to obtaining sample analysis, it can also be problematic because geology laboratories often have little, if any, experience in handling and processing biological samples. Also, they may perform only a small number of analyses daily, consistent with geological research needs. High-volume sample output as is needed in nutrition research may be difficult or impossible for these laboratories. Finally, non-nutritional laboratories may not provide much insight into interpreting the results of these studies and dealing with the specialized methodological issues described in this chapter.

For these reasons, we recommend that consultation be obtained with a researcher experienced in performing and interpreting results in planning a study. Even if the analysis is to be performed by a geology laboratory, this can be helpful to everyone, including the geologists.

16.5.2 Sources of purchasing the isotopes

Commercial isotope distributors are available for consultation and obtaining mineral isotopes. A few guidelines for working with distributors can be recommended. First, any distributor must provide complete information on the original source of the isotopes, their composition and any contamination. They should also be able to fully identify the pathway by which the isotopes were obtained. They should be prepared to have a sample tested if desired by an independent laboratory to assure the composition.

Once purchased, it is mandatory that end-users have batches of isotopes tested independently before human administration. This should be done in a laboratory experienced at these analyses.

16.5.3 Sample size for human studies

An important issue for researchers interested in this field is to identify the number of research subjects needed for participation. This number depends on the mineral, the variability in the expected results and whether a cross-over or a cross-sectional study is planned. With these key variables in mind, it is not uncommon for cross-over studies in which two conditions for absorption of the same mineral (e.g. iron) are being assessed to have adequate sample size with approximately 20–30 subjects. Studies involving calcium, zinc and magnesium may have slightly larger sample sizes. It is generally not appropriate to conduct studies with fewer than about 18–20 subjects in each group as variability in absorption is too great to have adequate power to identify a result.

16.6 Future trends

Three areas specifically relevant to functional foods will be considered. The first is the use of multi-mineral studies to evaluate the benefits of these products. Mineral stable isotope studies can readily be combined among minerals. It is not difficult to assess the nutritional effects of a dietary intervention or calcium, magnesium, iron and zinc simultaneously. The most important problems with such combined studies are dietary regulation and the actual administration of isotopes. It can be difficult to provide a fixed diet for 10 days to 2 weeks in which contents of all of the minerals are constant or relatively so. Also, it is not common to mix zinc or iron isotopes in a calcium-containing beverage. This requires some planning and consideration of the types of questions being asked before deciding how to give each isotope. It may be necessary to separate the meals or even the days in which the oral isotopes of different minerals are given in order to standardize the usual conditions for administration of each.

A related future trend is the intrinsic labeling of food products or meals with stable isotopes. This can be especially important for calcium. The solubility of the isotope and the way it is provided can affect the measured absorption. In some cases, there may be substantial error in providing an external tracer if a particular food effect is being studied (Heaney *et al.*, 2000). Intrinsic labeling refers to preparing the form of the mineral and sometimes the food with the desired chemical form of the isotope being evaluated (Weaver *et al.*, 1992). This can also be technically challenging but may be necessary for some studies.

The third topic is the performance of these studies in developing countries. It is increasingly recognized that functional foods may have key benefits for at-risk populations in developing countries. Specific benefits to the use of these products, such as increased iron and zinc absorption could be important from a public health perspective. However, as whole diets are very different in these settings, it is mandatory that hypothesized benefits

be tested in the populations at risk and using their usual diet. Remarkably, this is not difficult to accomplish. We have conducted or consulted in dozens of such studies. As an example (Avalos Mishaan *et al.*, 2004), we evaluated the effects of a novel micronutrient-fortified beverage on iron and zinc absorption in schoolchildren in a low-income urban area of Lima, Peru. We were able to utilize local resources to conduct the studies and collect samples, and then we sent the samples to the United States for analysis.

16.7 Summary and conclusions

Direct assessment of mineral absorption is possible in all population groups. Using stable isotopes, the effects of functional foods on the absorption of calcium, iron, zinc and magnesium can be identified. Therefore, it is not necessary to make assumptions about these effects, without supporting absorption data. Methodologies vary for each mineral, but are generally minimally invasive and well tolerated by children of all ages and adults. The availability of these methods may be used to support specific health benefits from functional foods and enhance their acceptance by individuals.

16.8 References

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